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— SECTION D —

ZOOLOGICAL SCIENCES

Contents

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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THE EXPRESSION AND INTERACTION OF HEREDITARY FACTORS AFFECTING HAIR GROWTH IN MICE: EXTERNAL OBSERVATIONS[1]

BY ARTHUR G. STEINBERG[2] AND F. CLARKE FRASER[3]

Abstract

Macroscopic observations on the expression of two mutant alleles ("hairless" and "rhino") in the house mouse, and on their interaction with one another and with a third mutation ("Naked") producing hypotrichosis of a different type, have been described. In hairless mice the juvenile pelage falls out and the skin shows little wrinkling; rhino mice lose their hair in a similar way but subsequently develop an intense wrinkling of the skin. Naked mice lose their hair by a breaking-off rather than a falling out, and show a pattern of alternate depilation and regeneration of the hair coat.

Hairless/rhino hybrids lose their hair according to the hairless pattern, but later manifest a characteristic wrinkling like, but not as extreme as, that of the rhino mutant. "rhino" is therefore not completely recessive to "hairless."

There are thus three basic grades of wrinkling—hr/hr, hr/hr^{rh}, hr^{rh}/hr^{rh}—determined by the factors present at the *Hr* locus. The presence of the heterozygous Naked factor in any of these types causes an intensification of its degree of wrinkling. This intensification is even more pronounced when the Naked factor is homozygous. Moreover, the "Naked" characteristics are themselves exaggerated in these compounds, the intensification being proportional to the degree of wrinkling.

These results will be interpreted in the light of histological findings to be presented in the following paper.

Introduction

The discovery by Howard (15) of a third mutation causing hypotrichosis in the house mouse made it possible to obtain new combinations of mutant factors of this type, thus affording an opportunity for further analysis of hairlessness in the mouse. Accordingly experiments were designed to investigate hypotrichosis by means of comparative studies of the morphology, both gross and microscopic, of the several phenotypes obtained and of the behaviour of their skins under conditions of changed environment brought about by transplantation. It was hoped that such a study might throw some light on how the genes concerned produce their effects and also on related problems of hair development and maintenance.

Hereditary hypotrichosis is of widespread occurrence, having been reported in various forms in most of the domesticated mammals. A comprehensive

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review of the literature on this subject has been presented by David (8). Cases reported since that time include occurrences as a recessive lethal in cattle (20, 24) and as a simple recessive in the cat (17, 18), in the rabbit (4, 10), in the rat (11, 21), and in the deer mouse (5). A review of the literature on hereditary hairlessness in mice is presented by Grüneberg (14).

The undertaking of a histological and experimental study of the development of the mutant types discussed in this paper was necessarily preceded by an examination of the external characteristics of these types. Macroscopic observations reported by previous investigators have been checked, modifications occurring in our stocks have been noted, and new compounds obtained by the writers are described. This paper presents these data.

Materials and Methods

The paper deals with three mutations, "rhino," "hairless," and "Naked," producing hypotrichosis in the mouse. The present "rhino" mutation, an autosomal recessive causing loss of the juvenile pelage and a subsequent characteristic wrinkling of the skin, was found in this laboratory by Howard (15). The "hairless" mutation used in the present study was first reported by Brooke (2) and further investigated by Crew (6, p. 335), Crew and Mirskaia (7), and David (8). It also is an autosomal recessive, allelic to rhino (15) and produces a loss of hair similar to that occurring in rhino mice, but little or no wrinkling. "Naked," an autosomal dominant, semilethal when homozygous, was first reported by Lebedinsky and Dauvart (16). David (8) has carried out a detailed macroscopic and histological examination of the Naked and hairless mutants.

Stocks carrying the mutants studied were being maintained in this laboratory when the present investigation was begun. Stocks used for observations of the mutant types were not closely related and had not been inbred prior to the beginning of the present experiments. Although there was some variation in the expression of the mutants studied, the differences between the various types were nearly always sufficiently great to afford accurate classification. Hence it was not considered necessary to inbreed the stocks for the purpose of reducing such part of this variation as was due to residual heredity.

Observations

rhino

Our observations on the rhino mutant types correspond, except for a few minor details, with those reported by Howard (15). Rhino mice grow a normal juvenile pelage but at about 14 to 15 days of age depilation begins in the region around the eyes and spreads out over the head and throat, and caudad along the back and belly. Hair loss starts later (18 to 19 days) but progresses more rapidly on the ventral surface than it does on the back. The body is usually denuded except for a few scattered hairs at 22 to 25 days. The hair on the feet, tail, and ears begins to thin out at this time and leaves

these parts completely bare by five to seven weeks of age. The eye-sensory hairs and vibrissae fall out prematurely but are replaced normally during the next growth cycle. They do, however, become fewer in number and more irregular in older rhino mice.

The pattern of hair loss is rather variable; it may range from a condition resembling the hairless pattern, with a fairly sharply defined wave of depilation progressing caudad and leaving relatively few scattered hairs in the denuded area (Fig. 1), to a generalized thinning of hair all over the body, with the anterior–posterior gradient poorly defined (as illustrated by Howard). The pattern often varies on the same mouse, being well-defined in the early stages, and becoming more diffuse as it progresses towards the posterior regions of the body.

A marked overgrowth of the nails occurs in rhino mice, beginning at about three weeks of age. By five to six weeks the nails begin to acquire the characteristic curved or spiral shape seen in older rhino mice. The teeth appear normal.

The wrinkling occurring in young rhino mice is the result of two different processes: (*a*) well-defined folds of skin, particularly in the neck region, occur in rhino (and normal) mice as the result of changes in the relative growth rates of the skin and the body during the growth of the first hair coat, and to some extent during the later periods of hair growth (9); (*b*) the wrinkling due specifically to the rhino condition begins to appear at about three weeks of age as a number of fine corrugations on the head, and to a less extent on the body. At five to six weeks of age the skin becomes noticeably thickened, the folds on the back of the neck are more pronounced than in normal mice of this age, and the corrugations on the head and back are coarse and prominent. At about nine weeks the characteristic lateral folds, running from fore leg to hind leg, make their appearance. By the age of six months all the manifestations of the fully developed rhino condition are present. The skin is intensely wrinkled and contains small, hard, white lumps, which increase in number and size with increasing age. The claws are long, and curved or spiralled, the skin folds over the eyes have developed to a point where the mouse is usually unable to see, and the lateral folds may be so enormous that they drag on the cage when the mouse walks (Fig. 2). When the skin of such a mouse is cut open and examined under the dissecting microscope it is seen to be filled with large numbers of round or oval vesicles of various sizes, which are filled with a thick, white, pasty material.

The fertility of rhino males seems normal, but that of the females is greatly reduced. Rhino females are also unable to suckle their young, which therefore have to be fostered.

"hairless"

Macroscopic observations on the development of the hairless condition correspond in general with those reported by David (8). Hairless mice differ from rhino mice in that: (*a*) depilation begins on the feet almost as soon as it

does around the eyes, and is complete by the time the dorsal wave of depilation has reached the ears (Fig. 3); (*b*) the line of demarcation between haired and non-haired regions is usually more sharply defined in hairless than in rhino mice; (*c*) there is a sparse regeneration of somewhat irregular and usually unpigmented hair at about five weeks, which falls out again about three weeks later and recurs at increasingly irregular intervals throughout life; (*d*) although hairless mice show various degrees of thickening and wrinkling of the skin with increasing age, they never approach the "rhinoceros" appearance of rhino mice of corresponding ages (Fig. 4). There is a hypertrophy of the nails similar to, but less extreme than that occurring in rhino mice.

The hairless mice in this laboratory, in contrast to those described by Crew and Mirskaia (7) and David (8), are fertile and vigorous, and females usually suckle their young, although their ability in this respect is rather subnormal.

Heterozygous "Naked"

The writers' observations on the expression of the Naked factor (both when heterozygous and homozygous) agree closely with those reported by David (8).

Depilation in heterozygous Naked (*Nn*) mice is the result of a breaking-off, rather than a falling-out, of the hair. The juvenile pelage is short and rough. Depilation begins around the eyes at about 10 to 12 days, spreads in a wave over the head and caudad along the body in a pattern similar to that of rhino, leaving many scattered hairs in the depilated areas (Fig. 5). The denuded skin (in non-albino mice) immediately after depilation contains many pigmented hair remains, which give it a dirty grey colour. These are gradually eliminated during the next few days. The feet remain haired at all times. At, or slightly after, the time when the dorsal wave of depilation has reached the tail (about three weeks) a new hair generation appears on the head and passes back along the body. Before this growth of hair has reached the tail, depilation has begun again around the eyes, and in this way more or less well-defined "waves" of hair pass anteriorly–posteriorly along the body at increasingly irregular intervals throughout life.

Homozygous "Naked"

In mice homozygous for the Naked mutation the vibrissae are absent at all times. The cycle of hair production in *NN* mice appears normal, but since most hairs either fail to erupt and remain coiled in the follicles, or break off

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EXPLANATION OF FIGURES

FIG. 1. *rhino, 19 days. Depilation rather more advanced than usual but feet are still haired. (Photograph by Howard.)*

FIG. 2. *rhino, 10 months. Note wrinkling of skin and overgrowth of nails. (Photograph by Howard.)*

FIG. 3. *hairless, 17 days. Feet are completely bare. (cf. Fig. 1.)*

FIG. 4. *hairless, six months. Note smooth skin, overgrowth of nails.*

PLATE I

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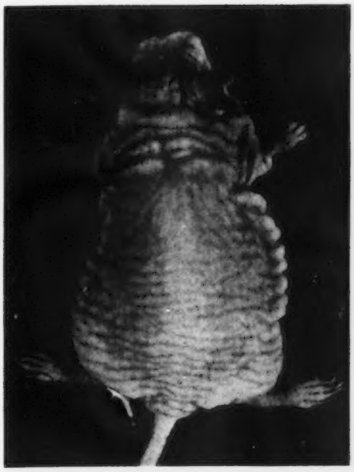

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shortly after reaching the surface, the animals remain almost completely depilated. The skin becomes highly pigmented during the periods of hair growth. The claws are soft and malformed. Such mice show a decided retardation in growth rate during the first few weeks of life, and usually die within the first 10 days after birth.

Tengbergen (23) reports a reciprocal difference in lethality of homozygous Naked (*NN*) young in crosses between *NN* and *Nn* parents, and of *Nn* young in crosses between *NN* and *nn* parents, the mortality in both cases being higher when *NN* mothers were used. She also asserts that there is a factor in the milk from *NN* females that has a detrimental influence on the coats of *Nn* and (fostered) *nn* young, and attributes both these effects to the presence or absence of a "B-substance" controlled by the *N* locus. However, her statement that the feeding and care provided by *NN* mothers can be neglected as a cause of the difference in mortality seems questionable—the difference in mortality among the *Nn* offspring of *NN* mothers (27 dead out of 81 mice) and the *nn* mice fostered on *NN* mothers (13 dead out of 56 mice so fostered) is of dubious significance—and it seems likely that her results may be explained simply by the fact that *NN* females are poor mothers and fail to provide adequate nourishment for their young.

hairless/rhino

Offspring of crosses of hairless × rhino mice (hr/hr^{rh}) lose their hair according to the hairless pattern, as described by Howard (15), and show a sparse regeneration of irregular, often unpigmented hairs over the body at about 40 to 50 days of age. However, in our experiments the rhino mutant is not completely recessive to the hairless allele as it appeared to be in Howard's (15), since at from six to nine weeks of age the hairless/rhino hybrids begin to develop a wrinkling that is characteristically rhino in pattern, but that is finer and less extreme than that of rhino mice of corresponding ages. The time at which these mice can be distinguished by their wrinkling from hairless mice of the same age is rather variable, but the difference has always been distinct by 9 to 10 weeks of age in the crosses observed by the writers. With progressing age the wrinkling of the skins of hairless/rhino hybrids gets coarser and thicker, and although it is never as intense as the wrinkling shown by rhino mice, such animals can always be distinguished from the normally smooth-skinned hairless animals (cf. Fig. 6, a hr/hr^{rh} mouse 180 days old, with Fig. 4, a hr/hr mouse of the same age). Hairless/rhino mice are fertile and are able to suckle their young.

Heterozygous Naked: Heterozygous rhino

Mice of the constitution $Nn;\ hr^{rh}/+$ were obtained from crosses of $Nn \times hr^{rh}hr^{rh}$ individuals. Observations made on those offspring of this cross that were phenotypically Naked showed that the rhino factor remains recessive to its normal allele when in combination with the heterozygous Naked mutation. There is no indication in these animals of "goggle" formation analogous to that occurring in $Nn;\ hr/+$ mice ((8) and present authors).

Heterozygous Naked; Homozygous rhino

Mice of the genetic constitution *Nn*; hr^{rh}/hr^{rh} were obtained by crossing *Nn* females with hr^{rh}/hr^{rh} males and crossing such of the F_1 animals as were phenotypically Naked (i.e. *Nn*; $hr^{rh}/+$) *inter se*. Of the six phenotypic types resulting from this cross (normal; heterozygous Naked; homozygous Naked; rhino, heterozygous Naked; rhino, homozygous Naked; rhino) those that were chosen as *Nn*; hr^{rh}/hr^{rh} showed the characteristics of both heterozygous Naked and of homozygous rhino mice. These mice were not homozygous Nakeds since they possessed vibrissae, and must therefore have been heterozygous Nakeds. They must also have been homozygous for the rhino mutation since it had already been shown that the heterozygous rhino factor did not express itself in the *Nn*; $hr^{rh}/+$ compound. As a check on the classification, several males of the type selected were out-crossed to normal females. The presence of Naked animals among the offspring of these crosses showed that the tested males were heterozygous for the Naked mutation.

Nn; hr^{rh}/hr^{rh} mice show a retardation in growth shortly after birth. The first hair coat may not appear until 8 to 10 days of age, and is very short and rough—much more so than that of *Nn* mice of the same age. Hair loss begins around the eyes at 12 to 13 days, and is usually complete except for a few scattered hairs by 22 days, leaving the skin with a dirty, smutty appearance due to the presence of pigmented hair remains (Fig. 7). No regeneration of hair occurs in these mice. There is an intense wrinkling of the skin during the period of depilation, and for several months wrinkling is more extreme than that in rhino mice. During the fourth or fifth month, however, the difference in wrinkling becomes less distinct, and eventually the two types can be distinguished only by the presence of numerous dark granules (evidently pigmented hair remains) in the skins of the *Nn*; hr^{rh}/hr^{rh} mice. There is no regeneration of hair after depilation has occurred.

Heterozygous Naked; hairless/rhino

Mice of the constitution *Nn*; hr/hr^{rh} were obtained by crossing *Nn*; hr/hr females with *Nn*; hr^{rh}/hr^{rh} males and selecting animals showing the short, rough coat characteristic of *Nn* mice before depilation.

As would be expected on the basis of the observations described above for Naked; rhino mice, the characteristics of both the hr/hr^{rh} and *Nn* types are also exaggerated in the *Nn*; hr/hr^{rh} compound. Depilation of the juvenile pelage is of the Naked type on the body, but, on the feet, hair loss occurs according to the hairless pattern, and by 15 days the skin on the feet is clean and pink. There is no subsequent regeneration of hair. Wrinkling is more

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EXPLANATION OF FIGURES

FIG. 5. *Naked, 19 days.*

FIG. 6. *hairless/rhino, two months. Note mild rhino-type wrinkling.*

FIG. 7. *Naked; rhino, 19 days. Note intense wrinkling, skin pigmentation.*

FIG. 8. *Naked; hairless, six months. Note mild wrinkling and pigment grains in the skin.*

PLATE II

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extreme than that in hr/hr^{rh} animals but less extreme than that in hr^{rh}/hr^{rh} mice during the first few months. In later stages (three to five months) wrinkling becomes typically rhino in character and degree, but the skin retains, throughout life, pigmented spots like those present in $Nn; hr^{rh}/hr^{rh}$ animals.

Homozygous Naked; hairless/rhino

One female of the constitution $NN; hr/hr^{rh}$ (offspring of a cross of $Nn; hr/hr \times Nn; hr^{rh}/hr^{rh}$) has been raised to maturity. This mouse was completely lacking in hair and vibrissae at all times, but the skin was intensely pigmented in the younger stages, and retained many dark granules throughout life. At the time of depilation, and for a few weeks afterwards, wrinkling was somewhat more extreme than that in rhino mice, but during the third month the difference became less and less distinct, and eventually disappeared.

Heterozygous Naked; Heterozygous hairless

An interaction of the Naked and hairless factors in which the characteristics of both mutant types are exaggerated in the compounds has been described by Snell (22) and David (8). The authors' observations on the interaction of the Naked and hairless mutations agree closely with those reported in these papers, but will be presented briefly here for purposes of comparison with other types. Mice heterozygous for both mutations ($Nn; hr/hr$) develop the characteristic "goggles" of hair around the eyes described by David—an interesting phenomenon in view of the completely recessive nature of the hairless mutation on a "normal" genetic background.

Heterozygous Naked; Homozygous hairless

Mice of the constitution $Nn; hr/hr$ show a retardation in growth shortly after birth. Their juvenile coat is short, sparse, and rough, and loss of hair may begin around the eyes as early as 12 days after birth. Depilation of the juvenile pelage on the body follows the Naked pattern, and leaves the skin moderately wrinkled. The feet, however, show the typical "gloves" of the hairless pattern. The skin remains distinctly wrinkled throughout life (to about the same degree as that of the hairless/rhino hybrids), and contains many dark granules, which can be clearly seen in Fig. 8.

Homozygous Naked; Homozygous hairless

One mouse of the constitution $NN; hr/hr$ (offspring of a cross of $Nn; hr/hr \times Nn; hr/hr$) has been observed. This mouse resembled the $NN; hr/hr^{rh}$ animal described in the preceding section. It was very small, completely lacking in vibrissae or other hair, and showed intense pigmentation and wrinkling of the skin. Unfortunately the animal died under anaesthesia while a piece of skin was being removed for histological examination, so no observations on later stages of its development were obtained.

Discussion

It may be seen from the above observations that the interaction of the Naked and rhino mutations is similar to that of the Naked and hairless factors—namely, an intensification in the compound of the characteristics of

each mutant type. Basically there are three grades of wrinkling, determined by the combination of factors present at the *Hr* locus; *hr hr* produces little, if any, wrinkling; hr^{rh} hr^{rh} gives rise to a pronounced wrinkling, while the hr/hr^{rh} compound is intermediate in this respect.

The presence of the heterozygous Naked factor in any of these types causes an intensification of the wrinkling in each case, and the Naked mutation in a homozygous condition shows an even more pronounced effect. Thus various combinations of the three mutants (hr, hr^{rh}, *and* N) form a series ranging from the smooth skinned *hr hr* to the extremely wrinkled $Nn; hr^{rh}/hr^{rh}$ type. In approximate order of increasing intensity of wrinkling they are: $hr/hr < hr/hr^{rh} = Nn; hr/hr < Nn; hr/hr^{rh} < NN; hr/hr = hr^{rh}/hr^{rh} < NN; hr/hr^{rh} < Nn; hr^{rh}/hr^{rh}$. (The $NN; hr^{rh}/hr^{rh}$ compound, in which wrinkling would probably be even more intense, has not been obtained.) This seriation is based on observations made during the first few months after depilation. In later stages, since there is evidently an upper limit to the amount of wrinkling the skin can display, in compounds in which the degree of wrinkling is extreme, the differences in wrinkling between the different types become less distinct with increasing age, and may eventually disappear.

On the other hand, the Naked characteristics are themselves exaggerated in these compounds, this intensification being correlated with the degree of wrinkling.

The discrepancy between the writers' observations on the hr/hr^{rh} hybrids and those of Howard (15) is very probably due to a change in the residual heredity of the hairless or rhino stocks or both between the times at which Howard and the writers made their observations (about three years). That the expression of the hairless and rhino factors is subject to alteration by genetic modifiers is not surprising in view of the range of variability shown by various strains of hairless mice in the past (e.g. the variation in degree of cyst formation described by David, and the differences in fertility, vigour, and suckling ability between the stocks of Crew, David, and the authors) and the fact that Crew and Mirskaia were able to improve the fertility of their stock by back crossing and selection.

Since the macroscopic observations reported above will be interpreted in the light of the histological findings to be described in the following paper, further discussion will be postponed until then.

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THE EXPRESSION AND INTERACTION OF HEREDITARY FACTORS PRODUCING HYPOTRICHOSIS IN THE MOUSE: HISTOLOGY AND EXPERIMENTAL RESULTS[1]

By F. Clarke Fraser[2]

Abstract

The first visible abnormality in the skins of homozygous rhino mice is a hyperkeratosis of the epidermis and follicle neck wall at the time when active growth of the hair ceases. This is associated with a widening of the hair canal (due to a lateral expansion of the hyperplastic layers of the follicle neck) and a subsequent irregular shortening of the follicle. The cause of hair loss is considered to be the widening of the hair canal and consequent lack of the support supplied by the normally tight-fitting follicle neck when shortening of the follicle raises the base of the hair to a level just below the proximal end of the hair canal. The hyperplastic tendencies of the epidermal derivatives are further expressed by (*a*) the development of hair canal cysts (utriculi), which leads to an increase in the surface area and a consequent wrinkling of the skin, (*b*) the formation of sebaceous-gland and follicle-end cysts, which cause the thickening of the epidermis, and (*c*) overgrowth of the nails.

Hairless (*hr hr*) mice show a similar, but less extreme, tendency towards hyperplasia, and the histological character of the skins of hairless/rhino hybrids is intermediate between that of the rhino and hairless types.

Mice of any of these types ($hr\ hr$, $hr^{rh}hr$, $hr^{rh}hr^{rh}$), which are also heterozygous for the Naked factor, show an exaggeration of both the follicular keratosis and the Naked characteristics. In mice homozygous for the Naked factor the exaggeration is more extreme.

Transplantation experiments show that rhino skin adjacent to normal skin behaves non-autonomously, indicating that rhino skin cells are able to utilize but cannot produce some substance necessary for the maintenance of normal stratified squamous epithelium; this substance is produced by normal skin cells but is not present in the blood stream of normal mice.

Results of an attempt to discover a relationship between the mode of action of the rhino mutation and the metabolism of vitamin A by feeding rhino mice massive doses of vitamin A were inconclusive.

Introduction

A consideration of the cases of hereditary hypotrichosis that have been studied histologically shows that the hair and skin respond to such mutations in a limited number of ways, the resulting histological patterns falling into a few well-defined classes. A review of previous studies on the histology of such conditions and the experimental attempts to understand or cure them may be based on such a classification.

1. A partial agenesis of the hair follicles, in which functional follicles do develop, but are greatly reduced in number, occurs in the rabbit (20, 8), in swine (40, 9), and in a normally hairless African rodent, *Heterocephalus* (45).

2. The primary abnormality may be a delay in the development of an otherwise normal follicle. Such a condition is reported by Mohr and Wriedt (27) in cattle.

[1] *Manuscript received August 8, 1945.*
Contribution from the Department of Genetics, McGill University, Montreal, Que.

[2] *Holder of Studentship from the National Research Council of Canada. Now in the R.C.A.F.*

3. Premature keratinization of the hair follicle and sebaceous glands may prevent the eruption of all except the guard hairs, as in the furless rabbit described by Castle (4) and Drapeau (11).

4. A condition in which imperfect keratinization of the hair shaft causes the hair to break off after its eruption occurs in the house mouse as the mutant "Naked" (8) and in the deer mouse (5).

David (10) showed that skin from a normal donor, when grafted on a heterozygous Naked host, behaved autonomously (one case). Treatment with benzyl mercaptan had no effect on hair growth in heterozygous Naked mice (10).

5. The juvenile pelage may fall out at the time of the first moult. Sometimes there is a partial regeneration of the hair coat, and there are varying degrees of cyst formation in the later stages. The mutant forms "hairless" (8) and "rhino" (18) fall into this group, which also includes a mutant type resembling "rhino" in the rat (37, 38, 48, 14, 41), and a mutant form of *Peromyscus* (44, 8, 5), which resembles the hairless/rhino hybrid to be described in this paper.

Certain human skin diseases, often showing a familial trend, and characterized by a follicular hyperkeratosis very similar to that observed in the above types, may also be considered in this category. These include Darier's disease, or keratosis follicularis (46, 31, 6, 25) and pityriasis rubra (3).

Most of the experimental work on hereditary hairlessness has been done on cases that fall into this class. Crew and Mirskaia (7) did epidermal transplants from haired to hairless adult mice, and succeeded in getting grafts to stay on with no loss of hair for a period of 14 to 30 days. David (10) obtained one successful graft from a hairless to a normal mouse; it behaved autonomously. Skin transplants from normal to hairless (39, 16), and from hairless to normal (16) rats also behaved autonomously. In all these experiments no attempt was made to inbreed the stocks used and transplant between closely related individuals, hence difficulty was experienced in getting grafts to survive in a healthy condition long enough to justify any definite conclusions about their autonomy.

Attempts to cure cases of hereditary hypotrichosis by treatment with various substances have met with little success. Martin and Gardner (26) fed hairless rats with cystine and cysteine and reported a regeneration of the hair coat, but Roberts (39), who repeated the experiment with cysteine, failed to stimulate any such regrowth. No explanation was offered for this discrepancy. Gershberg (16) fed cysteine to normal rats on which hairless skin had been grafted, again with no effect. The feeding of potassium iodide and of thyroid also failed to have any effect on hair growth in hairless rats (41). No significant difference between normal and hairless rats in weights of the pituitary, testes, spleen, or thyroids and no essential structural differences in the pancreas, thyroids, or adrenals were found (41).

David (10) treated hairless mice with benzyl mercaptan to test whether the loss of hair was due to a deficiency of the growth-stimulating sulphydryl group. There was no effect on hair growth, but in some mice there was a premature appearance and increase in number of hair canal cysts. This may have been due to irritation caused by the treatment rather than specifically to the growth-stimulating properties of the—SH group.

Several cases have recently been reported in the medical literature in which patients with Darier's disease (30, 47) and with pityriasis rubra (3) have responded favourably to treatment with massive doses of vitamin A. These cases all showed a low concentration of vitamin A in the blood or a high dark-adaptation level or both, indicating an inability of the affected person to absorb vitamin A from the intestinal tract, an inability to convert the provitamin (carotene) into vitamin A, or perhaps an increased demand for vitamin A.

A similar condition characterized by the appearance of spinous follicular papules, loss of hair, and plugging of the sebaceous glands can arise as the result of a deficiency of vitamin A in the diet (15, 29, 23, 24, 21). The pathology of the condition has been reviewed by Keil (19). There is a hyperplasia of the epithelium and a marked hyperkeratosis of the follicle opening, which is filled with a dense mass of cornified cells, occasionally containing the remains of atrophic hairs. The sebaceous glands atrophy, and there are degenerative changes in the sweat glands.

Moult (28) has shown that a vitamin A deficiency has a similar effect on the skin of rats, and Bessey (1) reports a metaplasia of other epithelial tissues, including those of the salivary glands, the respiratory tract, the genito-urinary tract and the eyes and para-ocular glands, to stratified squamous epithelium in vitamin A deficient rats.

6. "Hypotrichosis juvenilis," a condition described by Loeffler (22) in the house mouse presents characteristics of both Classes 3 and 5 in the above classification. There is the characteristic follicular hyperkeratosis, combined with a premature keratinization of the hair bulb. The striking thing about this abnormality is that it affects only the first hair generation. It would be interesting in view of the author's findings to be reported in this paper, to follow the transition from the abnormal first hair generation to the apparently normal second generation, but unfortunately Loeffler fails to describe this.

In a previous paper Steinberg and Fraser (43) described the external expression of the mutant types "rhino," "hairless," and "Naked" and their compounds. The present paper deals with a histological and experimental investigation of these types and with its contribution towards an understanding of the modes of action of the genes concerned.

Materials and Methods

For most of the histological work pieces of skin were removed from the mid-dorsum of animals that had been killed by severing the spinal cord at the base of the skull. In a few cases, when the animals concerned were needed

for breeding purposes or for further observation, a small piece of skin was removed under ether anaesthesia and the wound was sutured. Healing occurred within a few days, leaving a small scar. No observations that could have been affected by this procedure were made on such mice.

The skin specimens were fixed in Allen's B-15 fixative, embedded in paraffin, and cut in serial sections at various thicknesses, 10 and 20μ being found the most suitable. Sections were stained with Harris' modification of Delafields' alum-haematoxylin and counterstained with aqueous eosin. Heidenhain's iron-haematoxylin and Mallory's Azan triple stain were also used, and for cellular detail in the epidermal layers, where the cytoplasm is highly basophilic, a combination of Feulgen's nuclear stain with orange G and aniline blue was found useful. Further details of technique will be given, when necessary, in the following section of the paper.

For purposes of comparison with the mutant types to be described in this paper, a brief review of normal hair development based largely on the work of Dry (12) and Gibbs (17) is presented here. Follicle formation begins as a thickening of the basal layer of the epidermis, which develops into a cylindrical peg of epidermal cells extending down into the dermis. A concentration of dermal cells forms at the base of the young follicle and invaginates into it, forming the papilla. The epidermal cells surrounding the papilla form the follicle bulb, and cells proliferated from the latter region form the actual hair shaft, which is pushed upward through the follicle to the surface of the skin. At the same time the follicle itself elongates downwards, until about a week after the initiation of follicle development the follicle extends the full width of the dermis, and the layers of the inner and outer root sheaths are fully differentiated. The inner root sheath extends up only as far as the opening of the sebaceous gland duct; the region between this point and the surface of the skin is known as the hair canal and is lined only by the outer root sheath, which is continuous with the epidermis. The period from the beginning of development until growth ceases in the hair bulb is termed the Anagen phase (12).

The Catagen phase is marked by a rapid decrease in size of the hair root, the disappearance of the inner root sheath, and the formation of a broom-like hair club at the base of the hair shaft. The shortening of the root brings the hair club and surrounding sheath to a level just below the opening of the sebaceous gland duct where it remains throughout the next stage, Telogen, which is the latent period extending from the completion of the hair club until the initiation of the next Anagen phase (12).

Observations

Histological Observations

rhino

No histological abnormalities are seen in skin from the mid-dorsum of rhino mice until about 14 days of age, at which time hair loss is just beginning around the eyes and the hair on the back is in the late Anagen or early Catagen

phase (12), i.e. when pigment is ceasing to pass upward from the bulb and the follicle is commencing to shorten. At this stage the epidermis in rhino mice shows signs of hyperplasia, the germinal layers are well differentiated in contrast to those of normal skin, and the stratum corneum is thicker than normal (compare Figs. 1 and 2). The epithelium of the follicle neck also shows signs of abnormal growth activity and the upper part of the hair canal, which is enlarged, is filled with several layers of loosely packed squamous cells, whereas in the normal follicle at this stage the epithelium of the follicle neck is thin and poorly differentiated, and fits tightly around the hair shaft. Lower regions of the follicle neck are less affected, and at the base of the hair canal the follicle wall appears normal.

In non-rhino mice during the next three to four days the hair club is formed and shortening of the follicle brings the club, with its surrounding sheath, to a level just below the sebaceous glands (Fig. 3). In rhino mice at this stage, the widening of, and proliferation of squamous cells into the upper part of the hair canal continues, and extends down to the region of the sebaceous glands. Shortening of the follicle occurs irregularly, leaving elongated cords of epithelial cells extending down into the corium, and many of the hair clubs are malformed (Fig. 4). By 25 days the hair canals are over three times their normal width and only occasionally contain a hair. There is a marked hyperkeratosis of the surface epithelium, and cords of cells from the irregularly shortened follicles extend down almost to the panniculus carnosus (Fig. 5).

During the next few weeks enlargement of the hair canals continues, until in mice five to six weeks old they appear as large oval cysts, open to the surface, filled with masses of keratinized cells, and lined with stratified squamous epithelium. The sebaceous glands lie at the base of these "utriculi", or associated with the irregularly shortened follicle ends lower in the dermis (Fig. 6).

At about 30 to 35 days small vacuoles can be seen in the follicle ends, and similar vacuoles appear in the sebaceous glands shortly afterwards (Fig. 6). These vacuoles increase in size until by the age of 15 to 16 weeks they have developed into cysts as large as those developing from the hair canal cysts.

The follicle-end cysts contain varying amounts of a stratum-corneum–like substance and are lined with stratified squamous epithelium like, but not as thick as, that of the surface. The cells of the walls of the sebaceous cysts retain for a time their characteristic appearance, but eventually become

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Explanation of Figures

Fig. 1. *Normal, 13 days. Follicles in late Anagen, follicle neck close-fitting.* $\times$ *130.*

Fig. 2. *rhino, 14 days. Note follicular keratosis, widening of hair canal.* $\times$ *130.*

Fig. 3. *Normal, 18 days. Follicles in late Catagen.* $\times$ *190.*

Fig. 4. *rhino, 18 days. Note hair canal widening, hair club malformation.* $\times$ *190.*

Fig. 5. *rhino, 25 days. Note irregular follicle shortening.* $\times$ *115.*

Fig. 6. *rhino, 47 days. Showing typical utriculi, young sebaceous-gland and follicle-end cysts.* $\times$ *115.*

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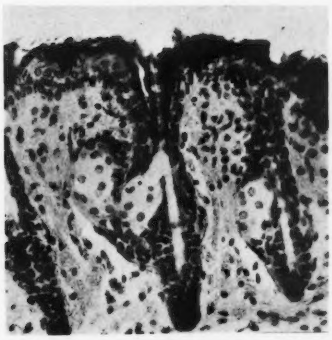

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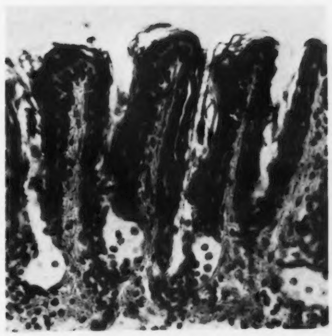

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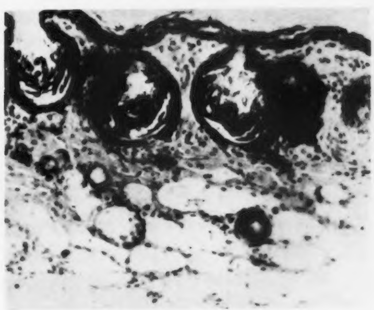

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elongated and compressed as if due to pressure within the cyst. Usually in the later stages the sebaceous and follicle-end cysts coalesce to form one large cyst.

At the age of seven to eight weeks the fat stores in the lower regions of the dermis begin to diminish, and by the age of 17 to 18 weeks they have disappeared (Fig. 7).

After the age of about three months the utriculi no longer increase and may even decrease in size, but the hair-follicle and sebaceous-gland cysts do continue to enlarge, until by 9 to 10 months they almost completely fill the dermis (Fig. 8).

hairless

In the skins of hairless mice a hyperkeratosis of the surface layers and follicle necks is, as in rhino mice, distinctly visible at 14 to 15 days (Fig. 9). Widening of the hair canals and irregular shortening of the follicles takes place in the next four to five days (Fig. 10). The hyperplasia of the surface epidermis and follicle neck does not, however, progress as rapidly as it does in rhino mice. The hair canal usually shows little or no increase in width beyond that reached at 18 days; in later stages the majority of the follicle necks are filled with a compact plug of stratum-corneum–like material and in relatively few follicles does hypertrophy continue towards the formation of a typical utriculus. The capacity for hair formation is often retained, perhaps because follicle organization is less interfered with than it is in rhino mice; such follicles produce hairs at the time when the second hair generation usually appears, but this capacity is progressively reduced with increasing age. These hairs may erupt through the hair canal, in which case they are usually very irregular in direction and shape, or occasionally they may break through the follicle wall and grow irregularly through the dermis. Davis (8) describes this process in detail.

The formation of follicle-end and sebaceous-gland cysts begins at 30 to 40 days as in rhino mice, but their subsequent enlargement and the consequent thickening of the skin is never as extreme as that occurring in rhino mice. In old hairless animals the walls of many of the cysts in the lower regions of the dermis are reduced to a single layer of squamous cells and such cysts become reduced in size and irregular in outline (Fig. 11). As in rhino mice there is a reduction and eventual disappearance of the fat deposits of the dermis in the skins of older hairless animals.

Naked

The author's observations on the dermal histology of the Naked mutant agree closely with those of David (8), who states that the condition is characterized by local defects in the keratinization of the hair shafts, which causes them to break off when the regions of weakness reach the skin surface. In some cases the weakness of the hair shaft is so great that the hair fails to penetrate the stratum corneum, but buckles within the follicle and may pierce the follicle wall and protrude into the dermis. In other cases the hair

may succeed in erupting, but in so doing the resistance of the stratum corneum may cause a considerable distortion and bending of the shaft, and a consequent irregularity in shape of the follicle. In regions where this results in pressure on the follicle wall, a mild hyperkeratosis may result (Fig. 12).

hairless/rhino

Histologically the hairless/rhino hybrids show an epidermal and follicular hyperkeratosis like that of rhino and hairless mice, but intermediate in degree between these types. In Fig. 13 it can be seen that the follicular hyperkeratosis in a 20 day old hairless/rhino hybrid is more advanced than that in hairless skin at 21 days (Fig. 10), but less extreme than that in rhino skin at 18 days (Fig. 5). In later stages the majority of follicles do produce hair-canal cysts, but smaller ones than those occurring in rhino mice of the same age.

Naked; rhino

The histological characteristics of both the Naked and rhino conditions are intensified in *Nn;* hr^{rh}/hr^{rh} mice. The follicular hyperkeratosis appears as early as the 12th day, progresses more rapidly than it does in rhino mice, and often includes part of the follicle wall below, as well as above the sebaceous gland. The bending and buckling of the hair shafts characteristic of the Naked condition is also exaggerated.

Skins of *Nn;* hr/hr^{rh} mice resemble those of *Nn;* hr^{rh}/hr^{rh} animals, except that the follicular keratosis and distortion of the hair shafts is less extreme (Fig. 14).

Transplantation Experiments

In order to decide whether the rhino condition is due to a specific effect of the rhino mutation on the skin cells, or whether it is a secondary manifestation of an effect on some other part of the body (e.g. the endocrine system), reciprocal ectodermal transplants between rhino and normal mice were performed. The technique used was that described by Reed and Sander (36). Grafts were done on the day of, or the day after, birth.

For the purposes of the present experiment it was necessary (*a*) that the grafts should be successfully incorporated on the host (for this reason the grafts were made between animals whose ancestors had been inbred brother-to-sister for several generations, so that the genetic constitution of the stock would be sufficiently homogeneous to allow compatibility between graft and host tissue), and (*b*) that graft tissue could be distinguished from host tissue

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EXPLANATION OF FIGURES

FIG. 7. *rhino, 4 months. Showing well developed cysts, disappearance of fat stores in dermis.* × *55.*

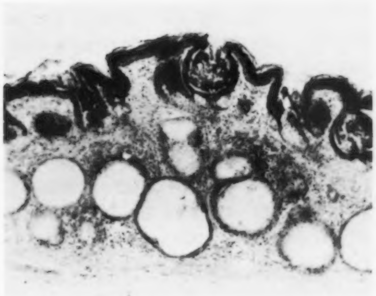
FIG. 8. *rhino, 14 months.* × *40.*

FIG. 9. *hairless, 15 days. Note follicular keratosis.* × *190.*

FIG. 10. *hairless, 21 days.* × *190.*

FIG. 11. *hairless, 15½ months.* × *40.*

FIG. 12. *Naked (Nn), 17 days.* × *85.*



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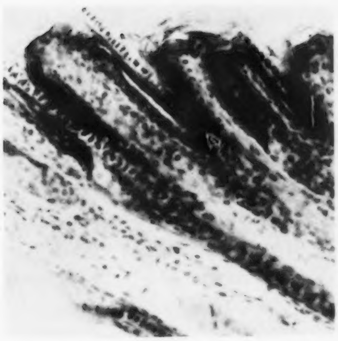

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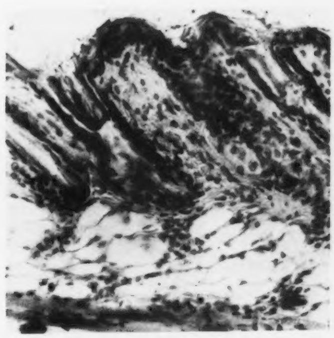

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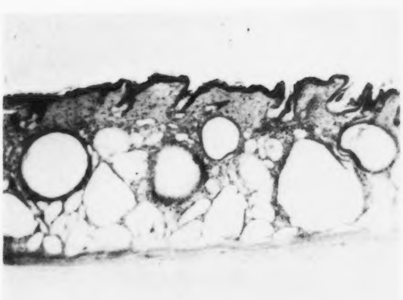

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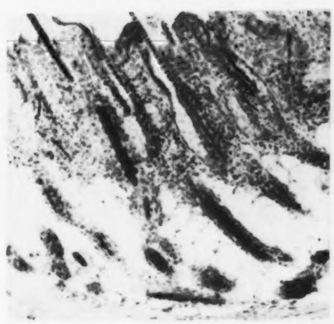

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at all times. To the latter end the "black-and-tan" mutant (a^t) was introduced into the rhino stock. Non-albino mice carrying this gene have a cream or tan coloured belly and a darker back, the colour of which depends on the other colour factors involved. Reed and Sander (36) have shown that this dorsoventral differentiation is determined early in embryonic life so that grafts of dorsal skin to a ventral environment and vice versa behave autonomously with regard to pigmentation except for occasional "invasion hairs," which receive at least their pigment cells from the host (33, 35). There is, moreover, a morphological dorsoventral differentiation, dorsal hairs being longer, coarser, and more closely spaced than ventral ones. Grafts from dorsal to ventral tissue behave autonomously in this respect also (33) with the possible exception of the "invasion hairs" mentioned above.

Black rhino males were crossed with females homozygous for the Black-and-tan gene and also carrying the albino (c) factor. F_1 animals were crossed *inter se* and, in following generations, black-and-tan rhino males were crossed with Black-and-tan heterozygous rhino sisters. Since the presence or absence of the rhino factor cannot be detected at birth, reciprocal grafts were made at random on the offspring of F_3 and later generations of this stock. Provided that both members of a pair survived, both host and graft could be classified as to their colour and whether or not they were homozygous rhinos at 15 days, at which time depilation has begun in rhino mice. In this way grafts from rhino to non-rhino, from non-rhino to rhino, from rhino to rhino, and from non-rhino to non-rhino mice were obtained.

Of the total of 74 grafts in which both members of a pair survived the operation there were only 25 that were considered completely successful "takes" (Table I, Column 1). These grafts showed little or no scab formation, grew a healthy coat of hair, and remained in good condition for two to three months or more. Eighteen grafts showed varying degrees of scab formation and replacement by host tissue, but retained patches of donor-type hairs in a healthy condition for several months. (Table I, Column 2). Eleven grafts grew a complete coat of hair but degenerated and sloughed off at the age of about one month (Table I, Column 3), while 20 grafts degenerated and

TABLE I

Donor	Host	1	2	3	4	Total
		Successful "takes"	Partial "takes"	Graft grew hair but fell off within one month	Graft fell off without growing hair	
rhino	non-rhino	5	3	0	7	15
non-rhino	rhino	4	4	4	2	14
rhino	rhino	5	8	5	3	21
non-rhino	non-rhino	11	3	2	8	24
Total		25	18	11	20	74

fell off without growing hair at all (Table I, Column 4). The most successful grafts seemed to be of ventral to dorsal or dorsal to dorsal tissue, but the numbers in each class were too small to permit conclusions regarding significance of the distribution. The failure of the unsuccessful grafts was probably due to the fact that there was still an appreciable amount of genetic heterogeneity in the F_4 and F_5 generations of the rhino stock, since Briggs and Jund (2), using a highly inbred strain, obtained 100% takes with a technique of skin transplantation that did not seem to differ essentially from that used by the author.

The grafting operation delayed hair growth on the transplant. Hair was not produced by the donor tissue for anywhere from 6 to 17 days after birth, the average time of appearance being about 12 days.

The operative procedure also delayed depilation in grafts of rhino skin to either rhino or non-rhino hosts. First signs of thinning on successful rhino grafts occurred at from 21 to 27 days of age, but usually at about 25 days. Thinning was first evident in the centre of the graft and the last hairs to be lost were those on the edges, regardless of the host's genotype. In the case of the successful rhino to rhino grafts these were gone by 41 to 49 days; their behaviour in rhino to non-rhino grafts will be described in detail later. In grafts of either rhino or non-rhino skin to rhino hosts there was also a delay in depilation of host hairs around the borders of the transplant; host hairs often remained around the borders of a graft for 30 to 45 days, well after depilation was complete on the rest of the body.

Successful grafts of non-rhino skin to rhino hosts behaved autonomously; they grew a normal coat of hair and showed no signs of rhino depilation. Fig. 15 shows a graft of skin from the back of a Black-and-tan non-rhino female that was transplanted at birth to the back of an albino rhino brother. The hair on the graft was still in good condition at the age of one year, when the mouse was discarded.

No effect of graft on host tissue or vice versa could be distinguished in this or similar cases. There was no delay in host depilation other than that attributable to effects of the operation as mentioned above. There was a narrow strip on the outer border of these grafts in which the graft hair was lost, but this was probably due to the formation of scar tissue in the zone of healing, and cannot be considered an effect of the rhino tissue on the graft.

EXPLANATION OF FIGURES

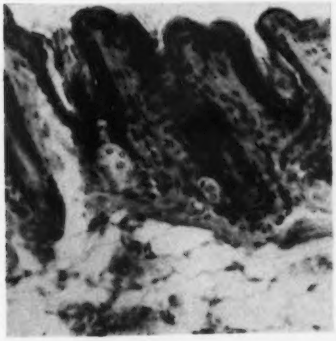
FIG. 13. *hairless/rhino, 20 days.* $\times$ *190.*

FIG. 14. *Naked; hairless/rhino* (Nn; $hr\ hr^{rh}$). $\times$ *85.*

FIG. 15. *Dorsal graft from Black-and-tan non-rhino to albino rhino litter mate, 52 days old.*

FIG. 16. *Ventral graft from Black-and-tan rhino to Black-and-tan non-rhino litter mate, 141 days old. Note tan hairs on border of graft.*

FIG. 17. *Section through edge of graft shown in Fig. 16. Normal tissue on right, rhino on left.* $\times$ *40.*



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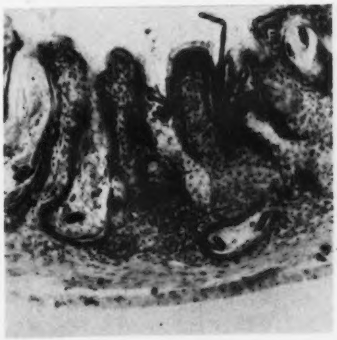

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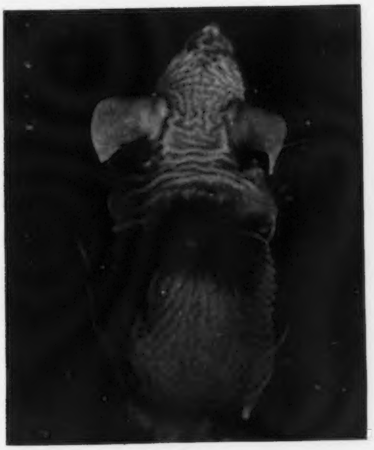

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Grafts of rhino skin to non-rhino hosts yielded results of an unexpected nature. In the case of ♀ 1313, for example, where a piece of ventral skin was grafted at one day after birth from a Black-and-tan rhino animal to the back of a Black-and-tan non-rhino litter mate, there was a luxuriant growth of tan hair all over the graft area by 22 days of age. During the next few days loss of hair began, first in the middle of the graft, and then spreading towards the sides. By 26 days there were only a few scattered hairs in the middle and a border of tan hairs around the edges of the graft. When this mouse died at the age of 10 months there was still a well-defined band of tan, ventral-type hairs on the right and left sides of the graft, a thin line of these hairs on the anterior edge, and a few scattered tan hairs on the posterior edge. A mild wrinkling and moderate thickening of the graft skin was detectable at about 35 days, but this never became as extreme as that occurring in rhino mice at corresponding ages (Fig. 16).

Histological examination of a small piece of skin removed from the edge of this graft at five and one-half months showed the characteristic rhino follicular and epidermal hyperkeratosis in the graft tissue, but this hyperplasia, and the development of the follicle-end and sebaceous-gland cysts, was less advanced than that in rhino mice of the same age. The thickness of the epidermal layers changed quite sharply in the transition zone between non-rhino and rhino skin, but the transition from normal follicles in the host epidermis to rhino type follicles in the graft tissue was somewhat more gradual (Fig. 17).

In a similar case, in which dorsal skin from an albino rhino male was grafted at birth to the back of a Black-and-tan non-rhino female, there were bands of white hair around the edges of the graft when the animal was discarded at the age of one year. In the three other cases in which successful rhino to non-rhino grafts were obtained, only irregular groups of graft-type hair remained around the border of the grafts but these groups have been retained for over seven months in the oldest mouse of this kind.

During the course of the grafting experiments some incidental observations were made concerning the effects of grafting operations on the morphology of the hair. It was noticed that both graft and host hairs, when situated near the borders of the graft, were often irregularly curved or bent, like the hairs of genetically "waved" mice. To test whether this "waviness" was due to alterations in the physical forces acting on the hair during its formation and growth, several grafts when placed on the hosts were rotated through 180° from their original direction.

The direction of the hairs on such grafts seemed to be affected by three factors: (*a*) a tendency of the hair to grow in its original direction, (*b*) irregular tensions set up in the skin as the result of scar tissue formation, and (*c*) a tendency for the hair to conform with the anterior-to-posterior direction of the host hair. Waviness of the hair was produced whenever there was an antagonism between any of these factors. In later months all the hair

on the graft tended towards an anterior-to-posterior orientation and the waviness gradually disappeared.

The fact that the operative procedure used in making ectodermal transplants can influence genetically "non-waved" follicles to produce phenotypically "waved" hairs throws considerable doubt on Reed's (34) interpretation of the results obtained when he grafted skin from waved to non-waved and non-waved to waved mice. He states that waved tissue influences the adjacent genetically non-waved hairs to become phenotypically waved. In the light of the above observations it seems clear that if there is such an influence of waved on non-waved tissue it will have to be demonstrated by some method other than that of transplantation.

Effects of Vitamin A

In view (*a*) of the histological resemblance of the follicular keratosis occurring in rhino and hairless mice to that produced in humans (19) and rats (28) by a hypovitaminosis A and (*b*) of the favourable response of several cases of hereditary follicular keratosis in humans to vitamin A therapy (30, 47, 3), it was decided to test the effect of feeding massive doses of vitamin A to rhino mice.

Unexpectedly the oral administration of from 10,000 to 40,000 units of vitamin A per day (one to four drops of ling liver oil containing 250,000 International Units of vitamin A per gram) produced an exfoliative dermatitis in young rhino mice, usually within a week, followed by a retardation in growth rate, weakness, emaciation, and eventual death after about one month. Young non-rhino mice showed a similar but less extreme effect accompanied by thinning of the hair coat. Treatment with a halibut liver oil concentrate containing 6400 International units of vitamin A per gram (the daily dose being between 2500 to 10,000 units) gave results less extreme but essentially similar to those produced by the ling liver oil treatment.

Adult rhino mice (two to three months or over) showed no visible effects of such treatment other than a desquamation of white powdery flakes from the skin of the head and body. Histological observations of skin from two such mice showed that the utriculi were smaller than those in untreated rhino litter mates.

Discussion

Histology

The first visible abnormality in the skin of both rhino and hairless mice is a hyperplasia of the stratified squamous epithelium of the skin surface and follicle neck and a widening of the hair canal, both of which are first evident at the time when the follicle has just started to shorten at the beginning of the Catagen phase. In normal follicles at the end of the Catagen phase (when shortening of the follicle brings the hair club to a level just below the sebaceous glands) the hair is evidently held in the follicle by: (1) the friction existing between the hair shaft and the cells of the close-fitting wall of the hair canal, and (2) the existence of an enlarged hair club at the base of the narrow cylinder in which the hair rests.

It is reasonable to suppose that in hairless and rhino mice the widening of the hair canal reduces the resistance offered to outward movement of the hair. It is the lack of support normally supplied by the tight-fitting follicle neck at the end of the Catagen phase that is considered to be the immediate cause of depilation. The subsequent irregular shortening of the follicle and malformation of the hair club are, in the author's opinion, probably a result of the disruption of the normal developmental processes consequent on the hyperkeratosis and widening of the follicle neck.

What causes the widening of the hair canal is not definitely known. The fact that the squamous cells are loosely packed in the hair canal, and do not look as if they were under pressure, indicates that it is not a result of a mechanical stretching of the follicle wall due to a crowding of the cornified cells into the canal. It seems more likely that the widening is due to an increase in the growth activity of the outer (i.e. distal to the hair canal) layers of the wall of the follicle neck, leading to a lateral expansion and consequent increase in circumference of the wall.

The author's conception of what constitutes the immediate cause of depilation does not agree with that of David (8) who attributes the loss of hair in hairless mice to the failure of the hair club to form properly. David does not mention any follicular keratosis previous to depilation and states that "following shedding, until new hairs are formed . . . the upper portion of the follicle appears normal in all respects." Unfortunately she does not illustrate the stages previous to depilation, but her illustrations of sections of hairless skin at the time of the first regeneration of hair (which is at about four to five weeks) show a definite follicular keratosis, which must have developed before this stage. Furthermore, the malformation of the hair clubs in both David's and the author's stocks is variable, and does not always seem great enough to appreciably reduce their "hair-retaining" powers.

Another point of disagreement with David (8) is in regard to the formation of hair follicle and hair canal cysts, which she considers to be due to "pressure exerted by the growing hair when its progress is impeded by some obstruction." This refers to hairs of the second generation, which, in hairless mice, develop in irregularly directed follicles and press against the sides of the hair canal during their progress towards the surface. In rhino mice hair-canal cysts develop in follicles that have never contained an irregularly shaped hair shaft; the shafts of the first generation hairs are straight, and rhino follicles have never been observed to produce hairs after the juvenile pelage is lost, yet cyst formation in rhino mice is much more extensive than in hairless mice. Utriculus formation in rhino mice is apparently an expression of the hyperplastic tendency of rhino epidermal tissue, and it is reasonable to suppose that the same applies in most cases to their formation in hairless mice, particularly since utriculi have been observed in follicles that showed no signs of hair regeneration. The formation of follicle-end and sebaceous-gland cysts is also thought to be due to this hyperplastic tendency. It is evidently not due simply to pressure inside the follicle resulting from the plugging of the hair

canal, since in cases where a vitamin A deficiency produces a similar follicular plug in rats there is no cyst formation in the lower parts of the follicle (28).

The difference in pattern of hair loss between rhino and hairless mice may be partially explained as follows: the time at which any individual hair is lost depends on (*a*) the rate at which widening of the hair canal occurs and (*b*) the time at which the hair club is formed and shortening of the follicle takes place. If the widening of the canal is relatively slight (as in hairless mice) the hair will probably not fall out until the end of the Catagen phase when resistance to outward movement is at a minimum. The pattern of hair loss will therefore correspond to the pattern in which the hairs of the juvenile pelage reach the Telogen phase, as diagrammed by Dry (12), and the boundary between haired and hairless areas should be relatively well-defined. If, on the other hand, there is a marked widening of the hair canal as in rhino mice it is likely that although the general pattern remains the same some hairs will fall out before shortening of the follicle is complete, resulting in a diffuse thinning of the hair coat instead of a sharp line of demarcation between haired and non-haired areas.

No explanation can be offered for the delay in depilation on the feet of rhino mice, since no observations were made on the behaviour of the follicles in this region. Also unexplained is the modifying effect of the heterozygous hairless gene on the heterozygous Naked factor resulting in the production of "goggles" in *Nn; hr/hr* mice, and the fact that the heterozygous rhino gene does not have any such effect.

As to the dominance relationships of the hr and hr^{rh} alleles, it seems that macroscopically the hairless factor is dominant to the rhino allele in the early stages, since hr/hr^{rh} hybrids lose their hair according to the hairless pattern and regenerate a sparse, fuzzy hair coat at about six weeks of age. In older hr/hr^{rh} mice, on the other hand, the rhino factor appears (in the present stocks) to be partially dominant, since a mild rhino-type wrinkling develops. Actually, however, the character of hairless/rhino skins is (as the histological observations have shown) intermediate between that of the rhino and hairless types at all stages. In this respect the rhino and hairless alleles are similar to the other multiple allele series in the mouse and in *Drosophila*. Further discussion of this problem at this time seems unwarranted.

The degree of wrinkling in the skins of mice carrying various combinations of the hairless, rhino, and Naked factors is directly proportional to the extent of utriculus formation, indicating that wrinkling is the result of an increase in surface area of the skin caused by the widening of the hair canals. Thickening of the skin, on the other hand, is evidently a result of cyst formation in the lower parts of the follicles.

The gradations of wrinkling in compounds carrying various combinations of the hr, hr^{rh}, and N factors are determined by: (1) a tendency towards hyperplasia of the epidermal tissues, the extent of which is determined by the mutant factors present at the Hr locus; hr^{rh}/hr^{rh} has the greatest effect, hr/hr has the least and hr/hr^{rh} is intermediate, and (2) an intensification of the

hyperplasia if such animals are carrying the *N* mutation, the exaggeration being greater in *NN* than in *Nn* mice. This exaggeration may be due to mechanical stimulation of the follicle walls by the irregularly bent or coiled Naked-type hairs (hypertrophy in response to irritation is characteristic of stratified squamous epithelia) but the retardation in growth rate and the intensification of the *Nn* characteristics before follicle widening takes place indicates an earlier interaction of the *hr* (or hr^{rh}) and *N* reaction chains.

Transplantation Results

It was recognized when these experiments were begun that the use of hair colour and form to distinguish graft from host skin was liable to possible complication owing to the occurrence in the grafts of "invasion hairs" which Reed (33) and Reed and Henderson (35) described as possessing host type pigment but determined as to their dorsal or ventral character by the origin of the graft, rather than by its position on the host. For instance, dorsal tissue from a Black-and-tan mouse grafted to the belly of a brown-and-tan host produced black hairs, with a few brown "invasion hairs" near the border of the graft. The brown hairs may have arisen either (*a*) by a migration of epithelial cells from the host into the graft and the formation by them of complete hairs that contained their own type of pigment but were dorsal in structure because they developed in dorsal-type tissue, or (*b*) by a migration of melanoblasts from the host into the graft and their subsequent entrance into dorsal-type follicles of the graft where they formed brown pigment according to their dorsal environment.

Although Reed and Henderson (35) do not present any conclusive evidence in favour of either possibility, they seem to favour the first interpretation. In the author's opinion the second is the better of the two. It seems more reasonable to suppose that melanoblasts are able to migrate through the dermis and become incorporated in a developing graft follicle than that a group of epidermal cells from the host pushes its way between the cells of the donor epidermis into the graft and then proceeds to form a complete hair, particularly since Rawles (32) has demonstrated that pigment-producing cells of the mouse epidermis can migrate extensively in the coelomic cavities of chick embryos.

For the purposes of the present discussion, however, it makes little difference which explanation one accepts for the presence of "invasion hairs" since at no time have they occurred with a frequency sufficient to create any difficulty in distinguishing graft from host tissue.

The grafting experiments reported above show that rhino epidermal tissues, when in close proximity to those of normal epidermis, behave non-autonomously. Apparently there is something in the epidermis (but not in the blood stream) of non-rhino mice that influences rhino epidermis adjacent to it to develop normally instead of according to the rhino pattern. The case of ♀ 1313, in which ventral skin from a Black-and-tan rhino mouse was grafted to the back of a Black-and-tan non-rhino sister, demonstrates this

clearly. The tan hairs on the borders of the graft did not fall out, although those in the central part of the graft did. The objection might be raised that these persistent hairs could have developed from cells that had migrated from the host tissue, but were ventral in nature because of their proximity to the ventrally organized graft tissues. That such is not the case is indicated by the fact that (*a*) the hairs around the graft borders developed as soon as, or sooner than, the other hairs of the graft; if they had been "invasion hairs" their appearance would have been delayed (33); and (*b*) in all the grafting experiments described by Reed or observed by the author the "invasion hairs" were present only as scattered individual hairs, never in well-defined rows as they were in the case of ♀ 1313. Moreover, in another case, where dorsal albino rhino skin was grown on the back of a Black-and-tan non-rhino host, if the hairs that remained on the edges of the graft had been "invasion hairs" they should have been black, instead of white as they were. This graft did actually show a few scattered black "invasion hairs."

The non-autonomous behaviour of rhino epidermal tissue when in close proximity to normal skin shows that the normal skin cells produce a cell-diffusible substance not present in the blood stream that is necessary for the maintenance of epidermal stratified squamous epithelium in its normal non-proliferative condition. The mutant epidermal tissues can evidently utilize the substance but cannot produce it, at least in quantities sufficient to keep the skin normal.

In view of the histological resemblance of the skins of rhino and hairless mice to those of mice suffering from hypovitaminosis A it was thought that the action of the *hr* and hr^{rh} mutations might be to interfere in some way with the metabolism of vitamin A by the cells of the mutant epidermis. Unfortunately, the treatment of rhino mice with massive doses of vitamin A gave very inconclusive results. The desquamative dermatitis, hair-thinning (in normal mice), and emaciation resemble the effects of feeding large doses of various vitamin A concentrates to rats (13, 42), but it is not yet certain that the toxicity of these concentrates is due to vitamin A itself.

The retardation in utriculus formation observed in rhino mice treated with vitamin A might possibly have been the result of a generalized retardation in body functions due to the treatment rather than a specific effect on the hyperkeratosis. Furthermore the number of mice observed was too small to rule out the possibility that the difference in utriculus size was due to random variation. A more extensive investigation along this rather promising line will be needed to settle these questions. Unfortunately this, as far as the author is concerned, will be impossible for the time being.

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